Reduced Diversity of AM Fungi in Annually Cropped Fields of the Canadian Prairie

C. Hamel, M. Dai, L. Bainard, H. Wang

Semiarid Prairie Agricultural Research Centre, 1 Airport Road, Swift Current, SK S9H 3X2

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Abstract

The arbuscular mycorrhizal (AM) fungi are important service providers to agriculture that are impacted by certain cultural practices. We assessed the status of AM fungal resources in the Prairie, from the Grey to the Brown soil zone, through sampling 176 wheat fields, 117 adjacent roadsides and 24 natural areas, at heading. The 18S rRNA gene sequences of AM fungi in soil metagenomic DNA accounted for 15% of all fungal target sequences recorded, and clustered in 122 operational taxonomic units (OTUs), a proxy for species. A few OTUs dominated under each land use type, and these OTUs often dominated under all three types of land use. The AM fungal community was influenced by land use and soil type. The size of the family Claroideoglomeraceae decreased from Black to Brown soils, and from roadside to field soil. On average, about eight OTUs were found in a field or a natural soil as compared to 14 in roadsides, and diversity (Shannon index) was lowest in cropland where the community was very uneven. The diversity and abundance of AM fungi was low in Brown soils. Lower AM fungal abundance and diversity in 2009, the dry year, than in 2010, the wet year, suggests that the proliferation of AM fungi in Brown soils may be limited by water availability. A long history of frequent fallow and wheat monoculture in the Brown soil zone may also explain reduced AM fungal diversity in these dry soils.

Introduction

Arbuscular mycorrhizal (AM) fungi can be seen as the probiotics of plants and soils. They improve plant nutrition and resilience to abiotic and biotic stresses, and divert carbon from photosynthesis into the soil system, improving soil structure and resilience to erosion (Smith and Read 2008). The AM fungi are known as biotrophs requiring the support of a living plant to exist. Thus, AM fungi may be negatively impacted in cropland, which is under plant cover for only three to four months per year whereas roadsides may constitute a good repository for AM fungal diversity. This study assessed the status and distribution of AM fungal resources in the Canadian Prairie.

Methods

In 2009 and 2010, soil and root samples were taken from 176 wheat fields and 117 adjacent roadsides or 24 adjacent natural areas at heading, in the main pedo-climatic zones of the Canadian Prairie, i.e., the Brown, Dark Brown, Black, and Gray soil zones. Root colonization was assessed by microscopic observation after clearing and staining root samples (Vierheilig et al. 1998). An approximately 250 bp region of AM fungal 18S rRNA genes was amplified from

soil metagenomic DNA using fusion primers AMV4.5NF/AMDGR (Sato et al. 2005) with multiplex identifiers. The amplicons produced were sequences in pools of 61 with Roche 454 FLX technology. Sequences were cleaned in Mothur, aligned in MUSCLE, and OTUs (a proxy for species) were clustered in Mothur and again in TOPALi. Shannon diversity index was calculated based on the abundance of AM fungal reads relative to all fungal reads obtained from each soil sample. The univariate data was analyzed by ANOVA and the multivariate data by perMANOVA. Means were compared by student-*t* tests following significant ANOVA.

Results

Soils in the difference soil zones differed in their levels of organic carbon; Black soils were richest and Brown soils poorest (data not shown). The level of AM root colonization of wheat followed the same trend, and varied from 13.5% in Black soils to 8.2% in Brown soils.

Fifteen percent of the fungal sequences detected were AM fungal sequences. On average, 613 good AM fungal reads were obtained per soil samples. A total of 122 AM fungal OTUs representing six families of AM fungi were obtained. Land use ($P < 0.0001$), year ($P = 0.0167$), and soil type ($P = 0.0292$) influenced the structure of the AM fungal community. These factors mainly impacted the size of the family Claroideoglomeraceae (Table 1). Roadside hosted higher abundance, diversity and richness of AM fungi than cropland (Table 1, Fig. 1). Overall, Dark Brown soils were found most hospitable to AM fungi, based on the abundance of AM fungal sequences, species richness, and Shannon diversity index (Table 1, Fig. 1). The dry 2009 season was less favourable to some AM fungal species than the wet 2010 season, as less species were detected that year, leading to lower Shannon diversity indices (Fig. 1).

Table 1. Abundance of AM fungal DNA sequences relative to all fungal sequences found in soils of different biomes and pedo-climatic zones, according to 454 pyrosequencing reads. Means followed by different letters are significantly different according to post hoc LSMean Student's *t* ($\alpha = 0.05$).

	Claroideoglomeraceae	Glomeraceae	Diversisporaceae	Paraglomaceae	Gigasporaceae	Archeosporaceae
Field	**4.57 b**	8.08	1.09	0.17	0.0014	0.00033
Natural	**5.55 ab**	7.63	1.22	0.18	0.0121	0
Roadside	**7.78 a**	8.89	0.72	0.07	0	0
Brown	**4.8 b**	8.59	0.62	0.102	0	0
Dark Brown	**5.76 a**	8.61	1.47	0.194	0	0.0007
Black	**7.69 a**	7.98	1.1	0.119	0	0
Gray	**4.99 ab**	7.72	0.57	0.114	0.016	0

Conclusion

Annual cropping shapes and reduces the diversity of AMF, but roadsides are a repository for the AMF diversity that may be lost in certain cropland. The Prairie population of AMF is impacted by drought, which suggests that dryness may explain the low abundance and diversity of AMF in

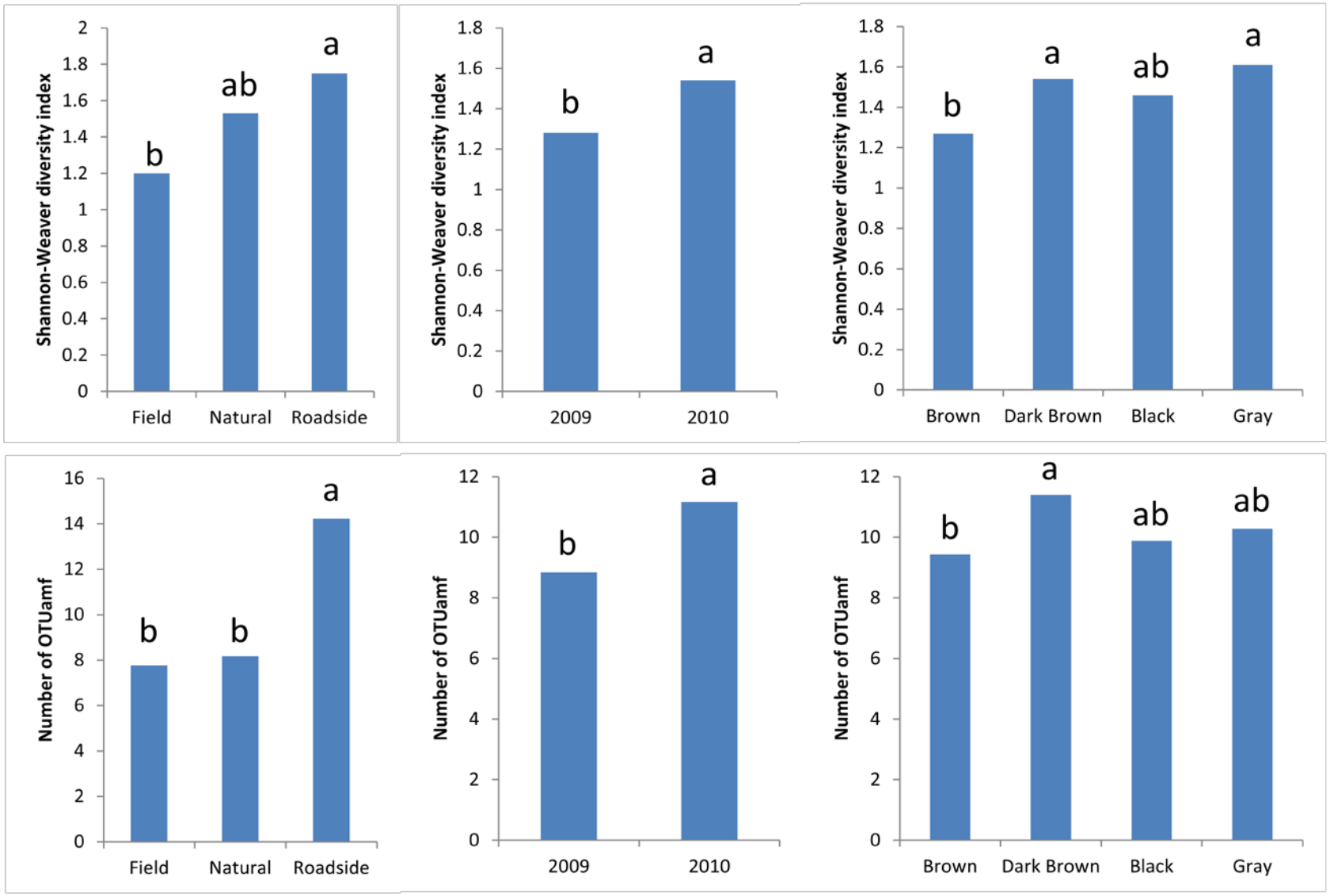

Figure 1. Level of diversity (Shannon index) and richness of the AM fungal communities as influenced by biomes, year and soil type. Bars associated with different letters represent different means, according to LSMeans Student's *t* ($P = 0.05$).

in Brown soils. The long history of frequent summer fallow and of wheat monoculture may also explain the low diversity of AM fungi in Brown soils.

This study is the first comprehensive study of the diversity of AM fungi in the Prairie Provinces. The consequence of the impact of crop production on the community of AM fungi in cultivated soils of the Prairie is unknown at this time. Experiments comparing the ability of different prairie-native AM fungal isolates that are on-going at SPARC, should clarify whether the AM fungal species selected under crop production are good, bad or fair mutualists in wheat and pulse crops.

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